siRNA-based therapeutics to treat liver diseases

Malu Martínez-Chantar

CIC bioGUNE
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LIVER DISEASE LAB

CONTENTS

About Us

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Principal Investigator of the Liver Disease Lab, CIC bioGUNE
Publications & 9 patents
Steering Committee CIBER
SAB, Pharmaceutical Companies and Research Centers
Woman in Hepatology

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MSc Management and Development of Biomedical Technologies
Publications

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The Liver Disease Lab

Magnesium accumulation upon cyclin M4 silencing activates microsomal triglyceride transfer protein improving NASH.

J Hepatol. 2021 Jul;75(1):34-45. doi: 10.1016/j.jhep.2021.01.043. Epub 2021 Feb 9.

Targeting Hepatic Glutaminase 1 Ameliorates Non-alcoholic Steatohepatitis by Restoring Very-Low-Density Lipoprotein Triglyceride Assembly.

Cell Metab. 2020 Mar 3;31(3):605-622.e10. doi: 10.1016/j.cmet.2020.01.013. Epub 2020 Feb 21.

Deregulated neddylation in liver fibrosis.

Hepatology. 2017 Feb;65(2):694-709. doi: 10.1002/hep.28933. Epub 2016 Dec 30.

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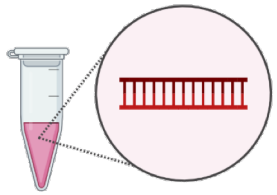
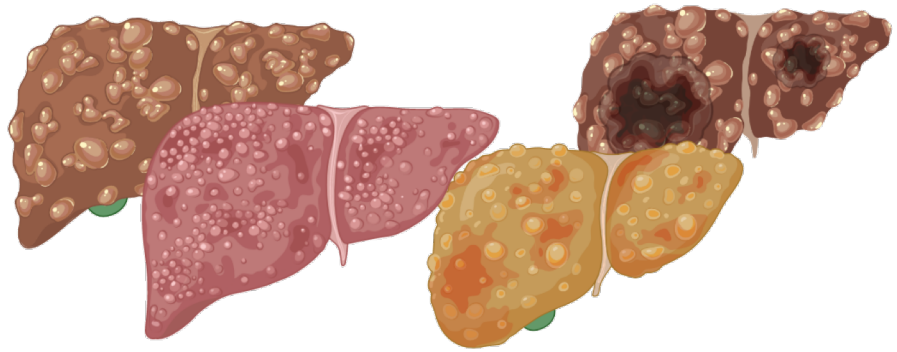
The Product

siRNA-based Therapy for liver diseases





Why?

Liver disease is an important cause of **morbidity** and **mortality** worldwide

29 million suffer from **chronic** liver disease in **Europe**

35 million suffer from liver disease in **USA**

2 million people **die annually** due to **cirrhosis and liver cancer**

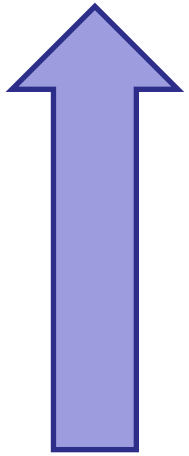
GLOBAL HEALTH PROBLEM

Chronic and acute **alcohol consumption**

New emerging risk factors: **obesity and type 2 diabetes**

Liver disease **market size** is estimated to reach 36,455 M$ by 2030

The value of the Liver disease treatment market size had a value of **20,673M$ in 2020** and is expected to grow at a **5.7% CAGR** from 2021 to 2030, accounting for **36,455M$.**



Growth factors:

- Unhealthy lifestyle and alcohol consumption.
- Rise in geriatric population globally
- Considerable rise in alcohol consumption is one of the major reason.
- Obesity.

Sources:
Allied Market Research
Transparency Market Research

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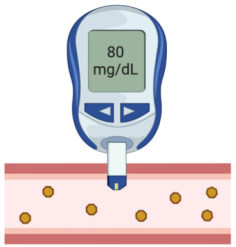
There is a need for innovative therapeutic solutions

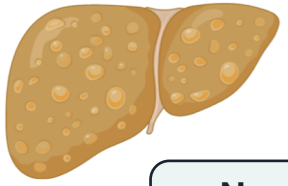
Our objective:

To find new polytherapeutic approach

Obesity

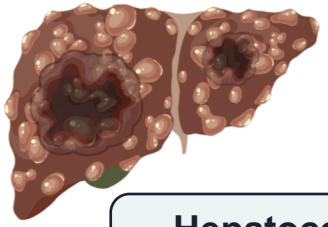


Diabetes

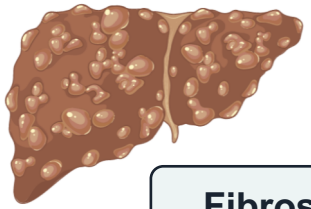


Non alcoholic steatohepatitis

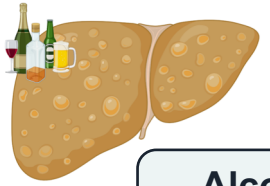
Magnesium (Mg^{2+}) homeostasis is **dysregulated in liver diseases**



Hepatocellular carcinoma (HCC)



Fibrosis



Alcoholic liver Disease (ALD)

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Intracellular Mg^{2+} concentrations are **tightly regulated by Mg^{2+} transporters**

Proteins	Tissue expression	Function
TRPM family		
TRPM6	Ubiquitous	Mg^{2+} influx
TRPM7	Ubiquitous	Mg^{2+} influx
SLC41A1		
SLC41A1	Ubiquitous	Mg^{2+} efflux
SLC41A2	Ubiquitous	¿?
SLC41A3	Ubiquitous	Mitochondrial Mg^{2+} efflux
MRS2	Ubiquitous	Mitochondrial Mg^{2+} influx
MagT1	Ubiquitous	Glycolisis
MMgT1	Ubiquitous	Membrane anchoring
CNNMs family		
CNNM1	Brain, testis	Mg^{2+} efflux
CNNM2	Kidney	Mg^{2+} influx and efflux
CNNM3	Kidney, brain, lung	Mg^{2+} influx
CNNM4	Intestine	Mg^{2+} efflux

AIM

Target the CNNM family of **Mg^{2+} transporters** to **restore Mg^{2+} homeostasis** and alleviate the progression of liver diseases

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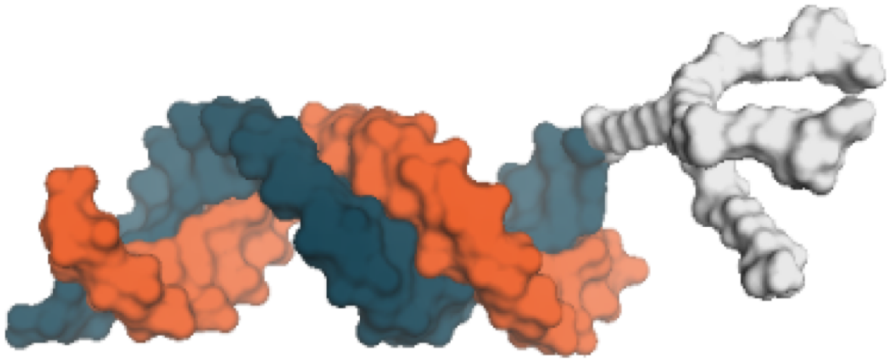

siCNNM4-GalNAc (MCH002)

Our Product

More tan 4000 sequences tested

- Subcutaneous administration
- Low Dose
- Stable
- No off target effects
- Hepatocyte Specific

Other Subcutaneous injection, approved by the FDA and EMA for clinical studies

- ONPATTRO®, GIVLAARI™ from Alnylam
- Others in Phase III: Lumasiran (Alnylam), DCR-PHXC (Dicerna), QPI-1002 (Quark Pharma)…
- Low dose (~ 200 mg/month in humans)

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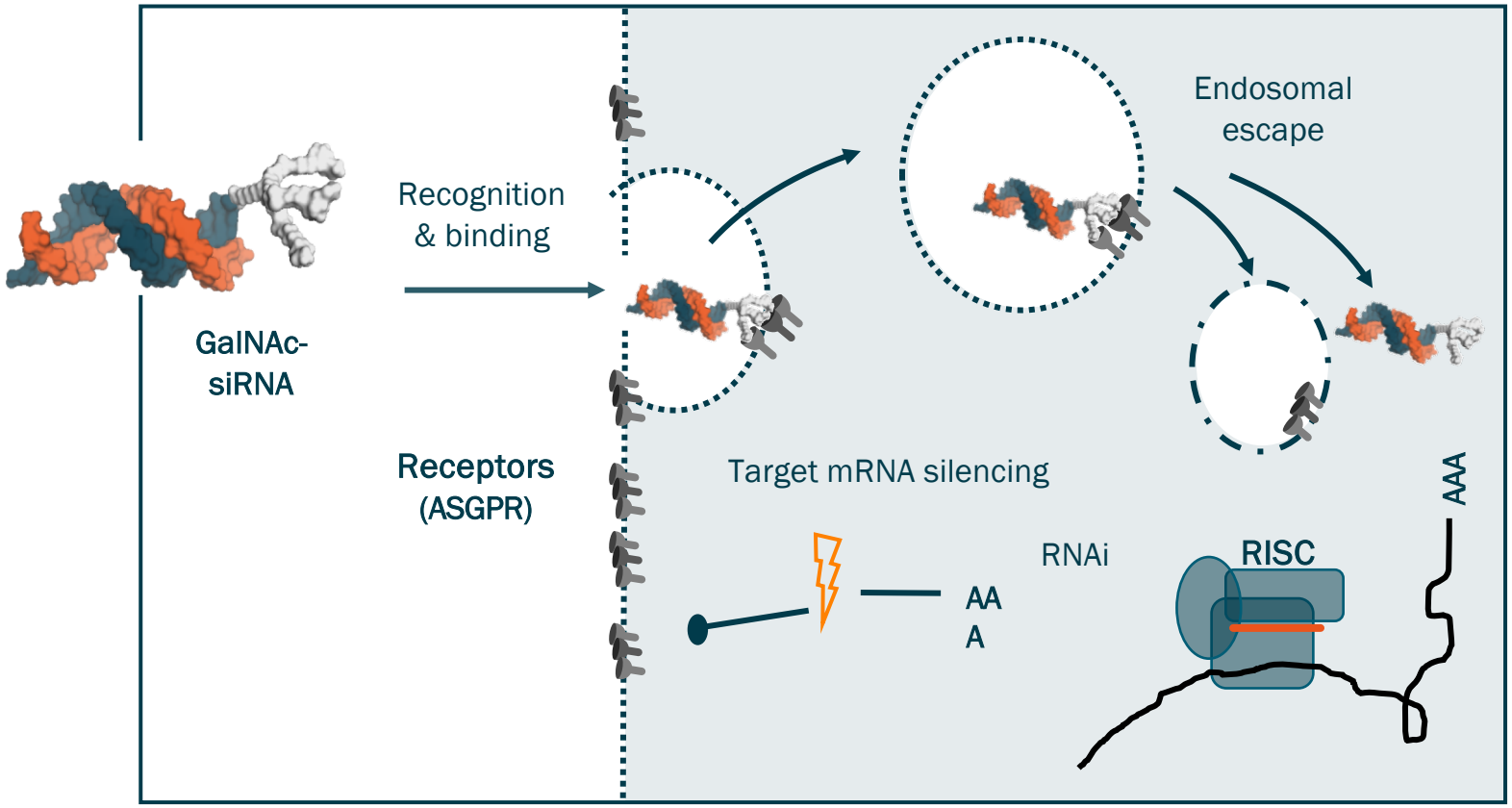
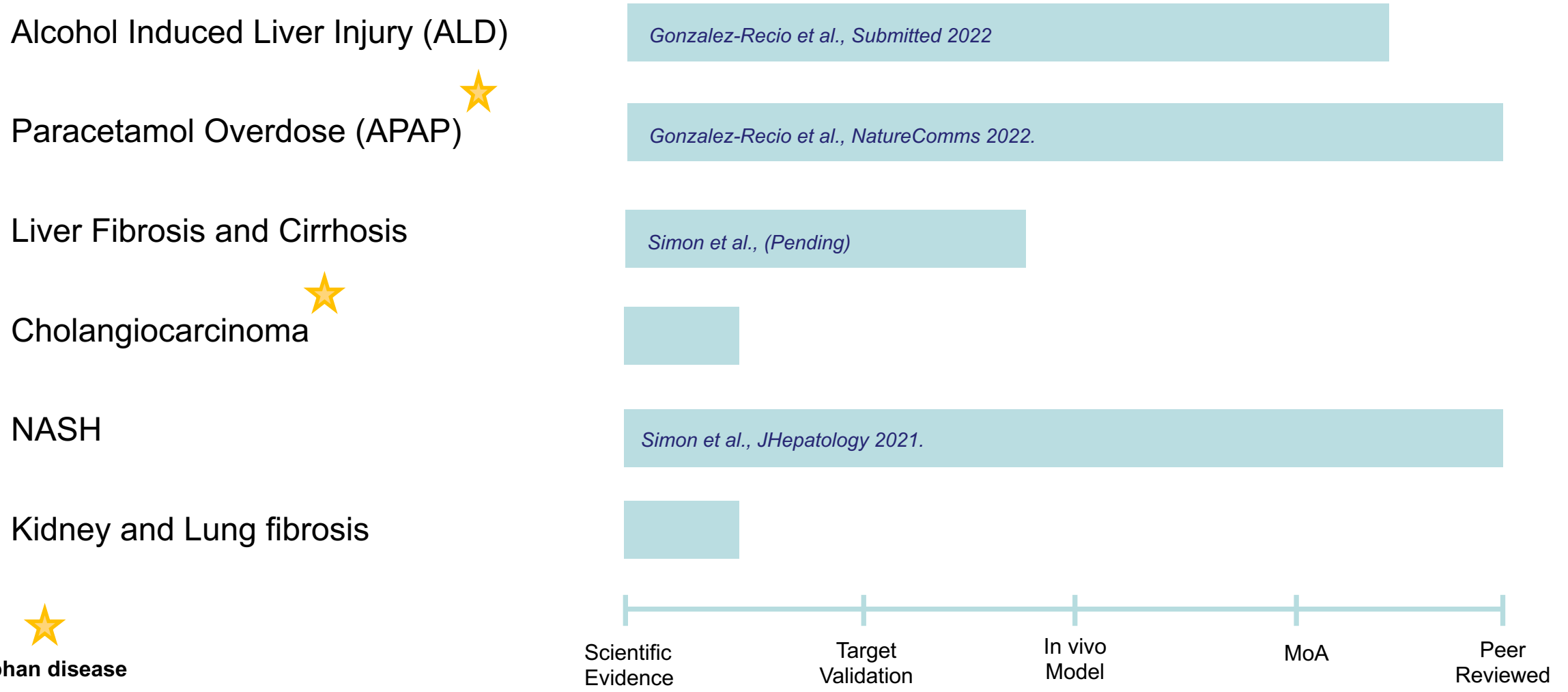
N-Acetylgalactosamine (GalNAc-building blocks-siRNA)



Validation of **Mg^{2+} homeostasis impairment** in other pathologies **where** the crosstalk between **ER and mitochondria** is **compromised**, such a **Drug induced Liver Injury and Alcoholic liver disease (from hepatitis to cirrosis)**

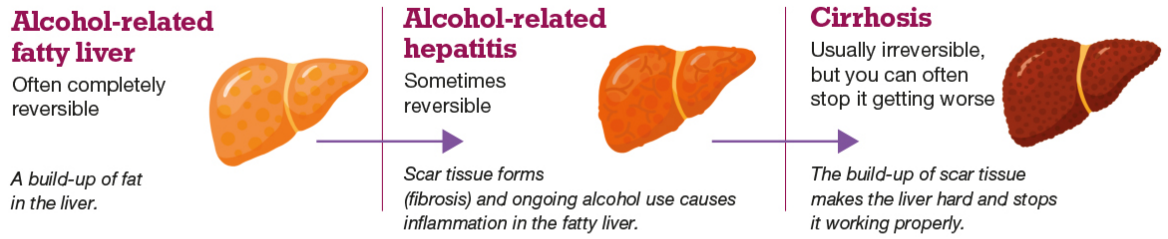
Preclinical Validation for MCH-002



Alcohol induced liver injury (ALD)

1st Indication Use Case

- **Alcohol abuse** is one of the leading causes of **chronic liver disease** and **liver-related deaths** in Western countries.
- The OMS has reported that **50% of liver cirrhosis cases** are due to alcohol abuse, causing **3.3 million deaths**.
- **Consumption** is **expected to increase**, along with its complications



Source:
British liver Trust

Currently, **there are no effective treatments** for this disease, except **abstinence** or **liver transplantation.**

Current treatments

- Alcohol Withdrawal Treatment
- Nutritional Support
- Liver Transplant
- Treating Complications of ALD

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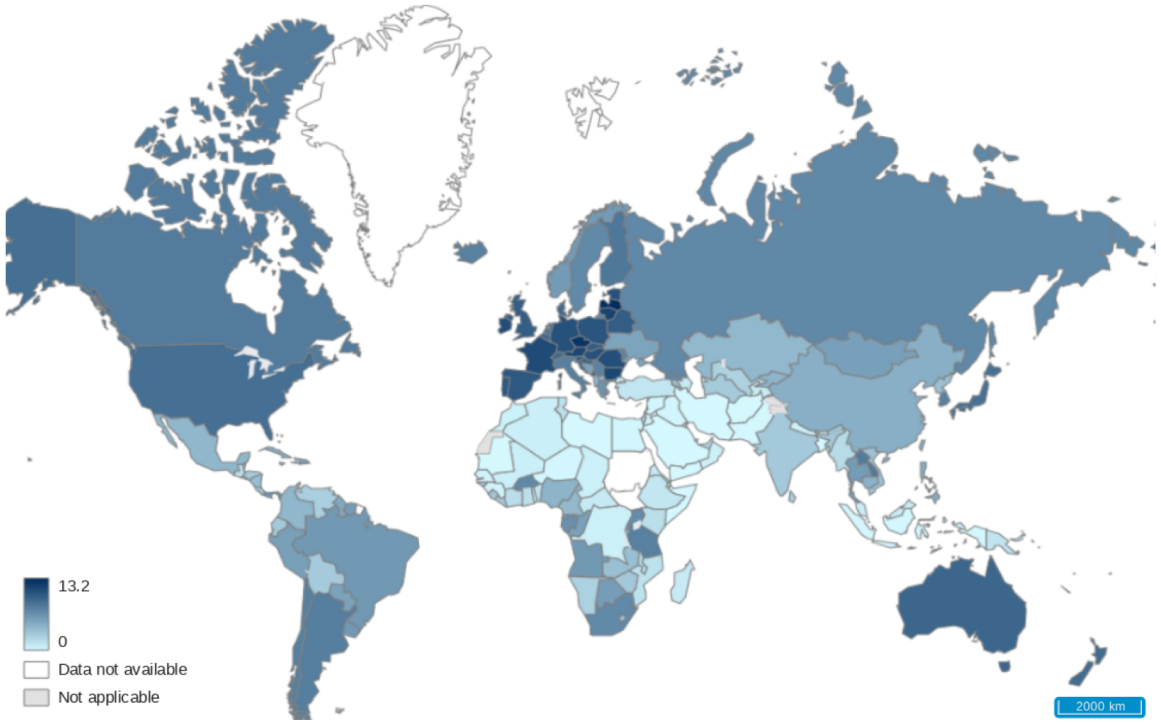
Alcohol consumption per capita (+15years, liters of pure alcohol) in 2018



Harmful use of alcohol

3 million

people died globally in 2016

Alcohol consumption

57%

of adults abstained from alcohol in the past 12 months, in 2016

National alcohol policy

46%

of Member States reported having one in 2016

Sources:
WHO: Global Information System on Alcohol and Health

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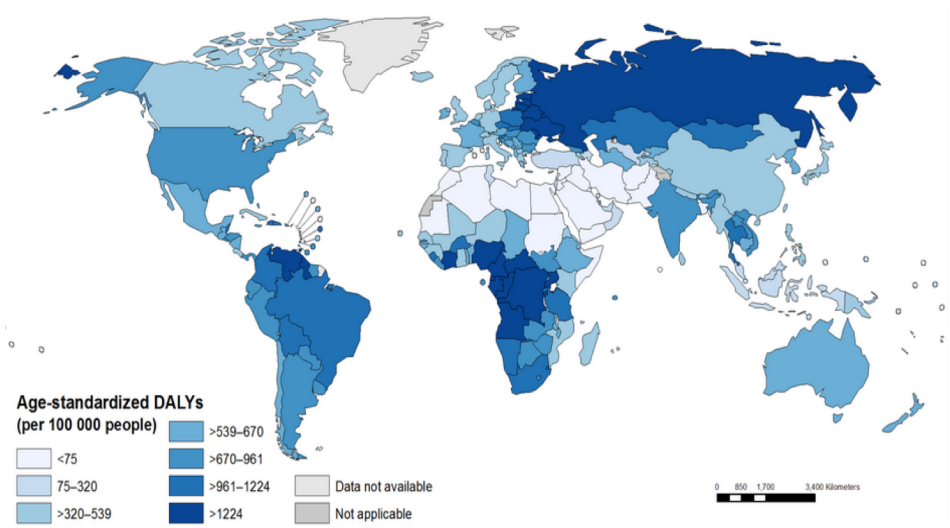
Alcohol-attributable injury **Disability Adjusted Life Years** (DALYs) in 2016



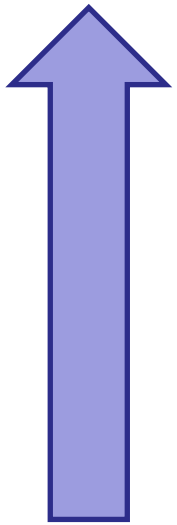
In 2016, alcohol led a large burden of disease and injury, causing **132.6 million DALYs** which represented 5.1% of all DALYs in the year

Across Europe, prevalence and mortality data indicate that **increasing rates of cirrhosis** in Europe are linked to dramatic **increases in alcohol consumption**, most notably in Northern European countries.

- **Costs** related to alcohol consumption have been estimated at **125 billion euros** in the European Union in 2003 and **249 billion dollars** in the United States in 2010.
- The **prevalence** of alcoholic fatty liver has remained stable from 2001 to 2016, affecting 4.7% of adults in 2015-2016. However, the prevalence of fibrosis with stage 2 or cirrhosis (stage 3) **increased significantly**, affecting 1.5% and 0.2% of adults, respectively, in 2015-2016.
- The highest percentage in 2016 of overall **deaths attributable to alcohol consumption** was in Europe at **10.1%.** The **burden** of acute liver diseases (ALD) in Europe is considerably higher than in the United States and is estimated to be the **highest in the world**.

Sources:
WHO: Global Information System on Alcohol and Health
Richardson, C. T., & Singal, A. K. (2018). Epidemiology of alcoholic liver disease. *Clinical Epidemiology of Chronic Liver Diseases*, 75–98 (*DOI: 10.1007/978-3-319-94355-8_7*)

The value of the Alcoholic Hepatitis **market is expected to grow** at a **6% CAGR** from 2019 to 2030, reaching a total value of **1.2Bn$.** In terms of **treatment, the corticosteroid segment** is likely to dominate the global alcoholic hepatitis therapeutics market.



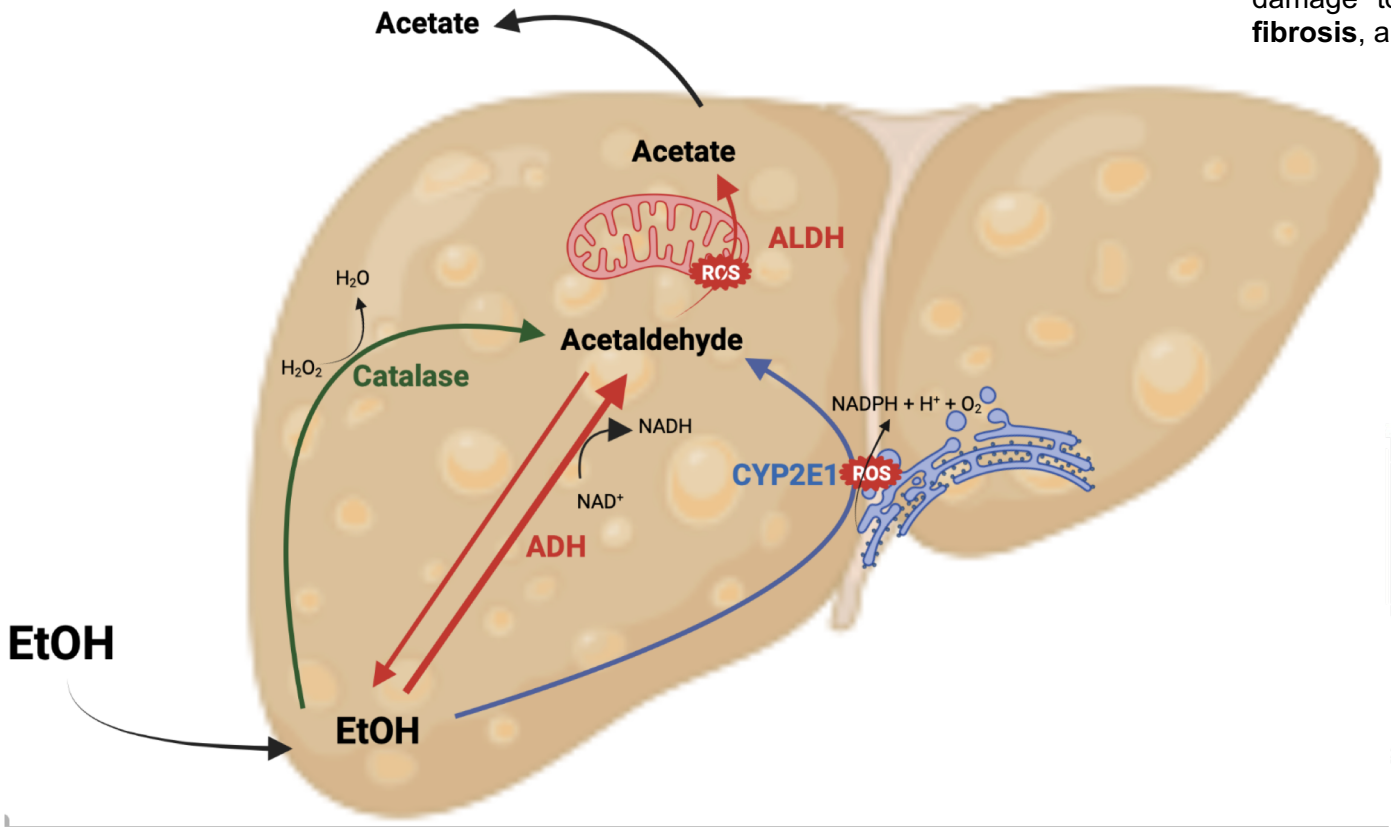
Growth factors:

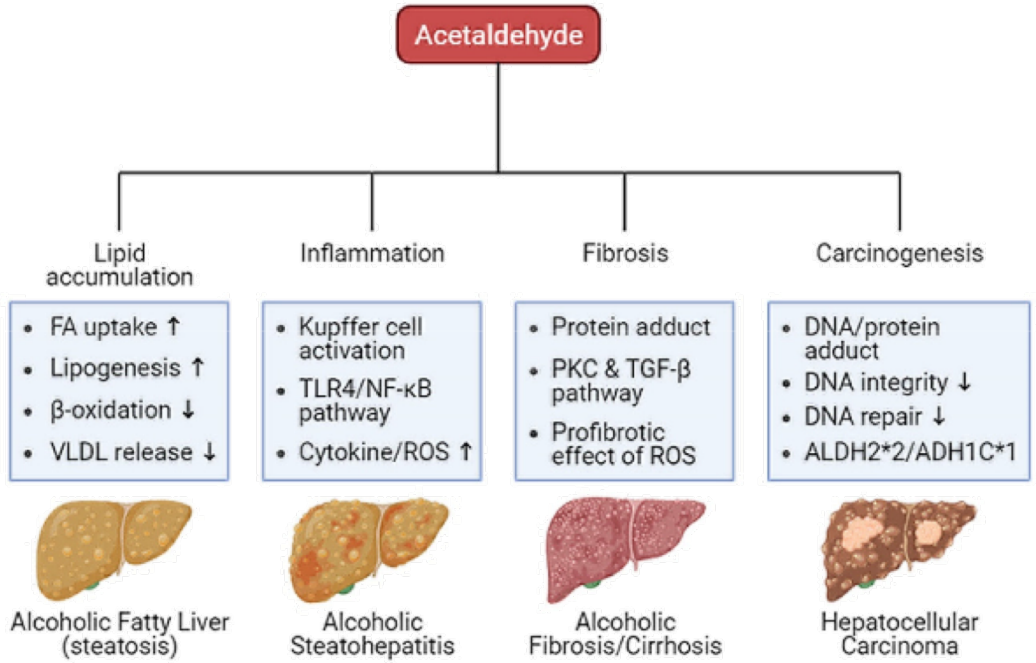
- Rise in number of alcohol related diseases worldwide.
- Widening knowledge related to Pathophysiology.
- Rich product pipeline to treat alcoholic hepatitis

Sources:
Allied Market Research
Transparency Market Research

Ethanol Metabolism

Acetaldehyde, one of the oxidative ethanol-derived metabolites, exerts a broad spectrum of damage to the liver, ranging from **lipid accumulation** in hepatocytes to **inflammation, fibrosis**, and **carcinogenesis**. ROS-mediated **oxidative stress** also accelerates liver fibrosis.





Ceberbaum A.I. Clin. Liver. Dis 2012

Hyun, J., Han, J., Lee, C., Yoon, M., & Jung, Y. (2021*). Pathophysiological Aspects of Alcohol Metabolism in the Liver. International Journal of Molecular Sciences, 22(11)*

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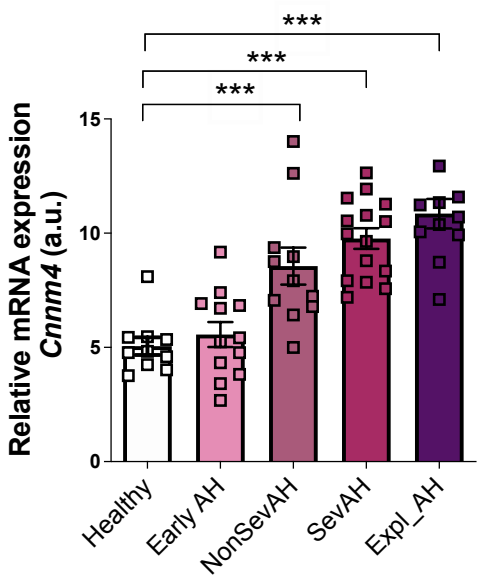
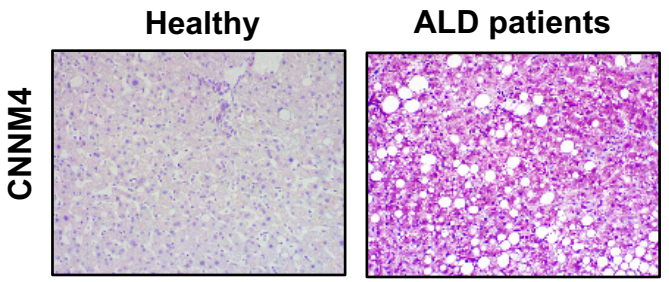
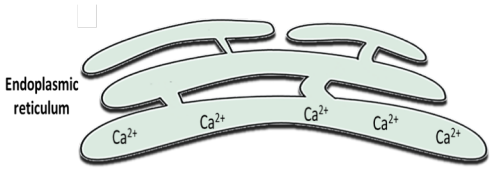
Hepatic CNNM4 expression is induced in ALD which is also correlated with Mg^{2+} serum levels





Targeting *Cnnm4* in ALCOHOL HEPATITIS is able to overcome Mitochondrial dysfunction

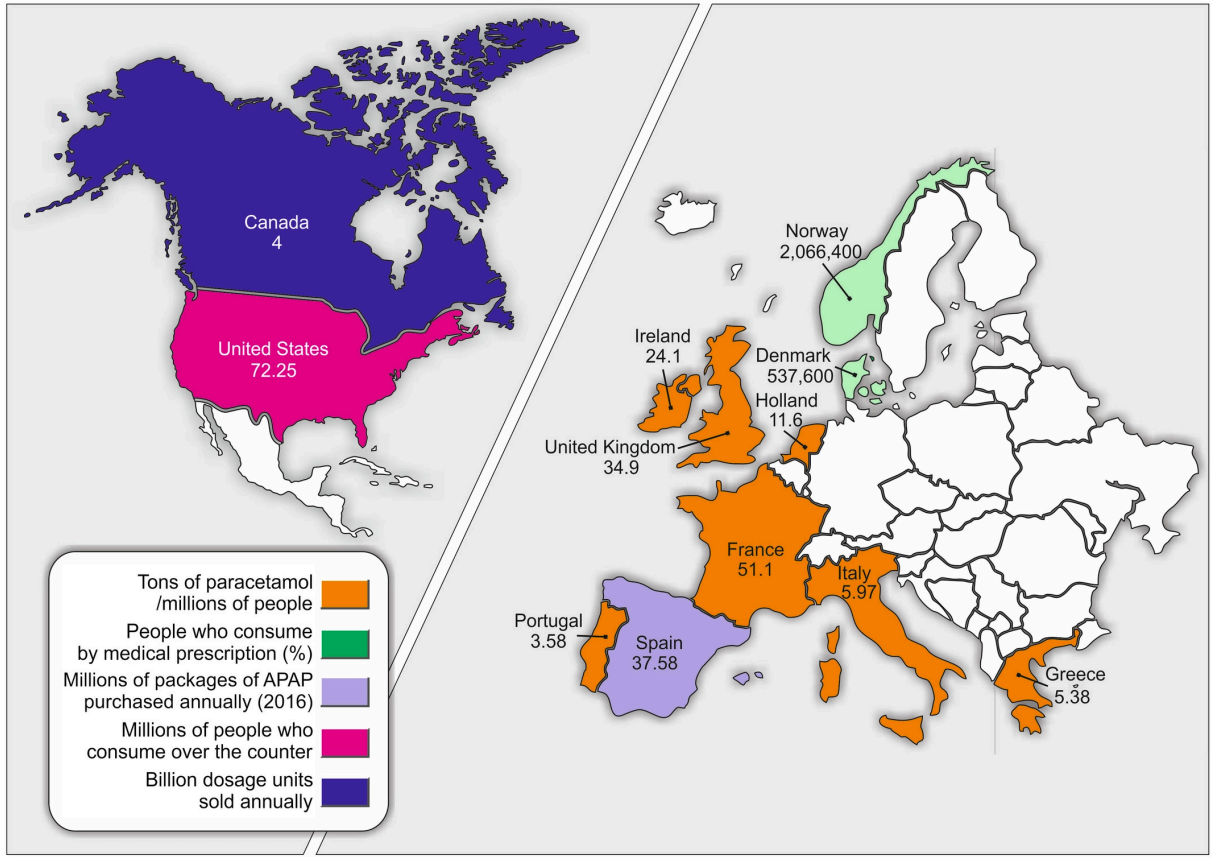
Targeting *Cnnm4* in ALCOHOL HEPATITIS is able to overcome ER stress in the liver



Targeting *Cnnm4* in ALCOHOL HEPATITIS is able to induces the REPAIRING activity of PIMT in the liver

APAP induced liver injury

- **Estimated to affect 19 out of 100,000 citizens worldwide**
- **Paracetamol (APAP) overdose** represents 50% of cases of ALF in the USA
- **About 30000 patients with APAP-related liver injury** are admitted to intensive care units annually**, and 29% of them require liver transplant**
- One of the therapies available today is **N-acetylcysteine,** which is effective only in the first few hours of APAP overdose.



García Roman et al. Clinical Pharmacology and Therapeutics 2019

Andrade, R. J. *et al.* Drug-induced liver injury. *Nat. Rev. Dis. Prim.* **5**, (2019).
Lee, W. M. Acetaminophen (APAP) hepatotoxicity—Isn't it time for APAP to go away? *J. Hepatol.* **67**, 1324–1331 (2017).
Bernal, W., Auzinger, G., Dhawan, A. & Wendon, J. Acute liver failure. *Lancet* **376**, 190–201 (2010).
Fisher, E. S. & Curry, S. C. *Evaluation and treatment of acetaminophen toxicity*. *Advances in Pharmacology* vol.

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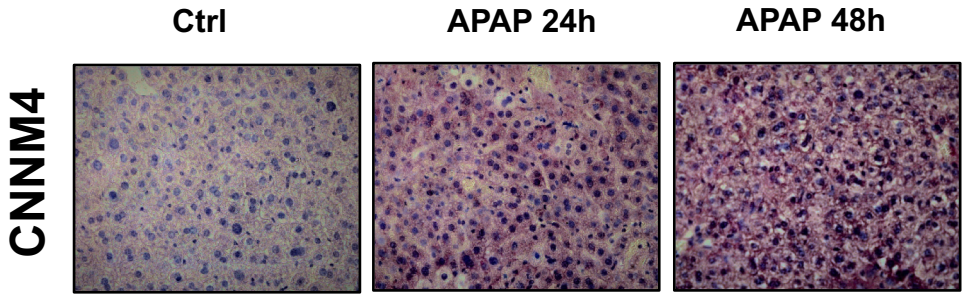
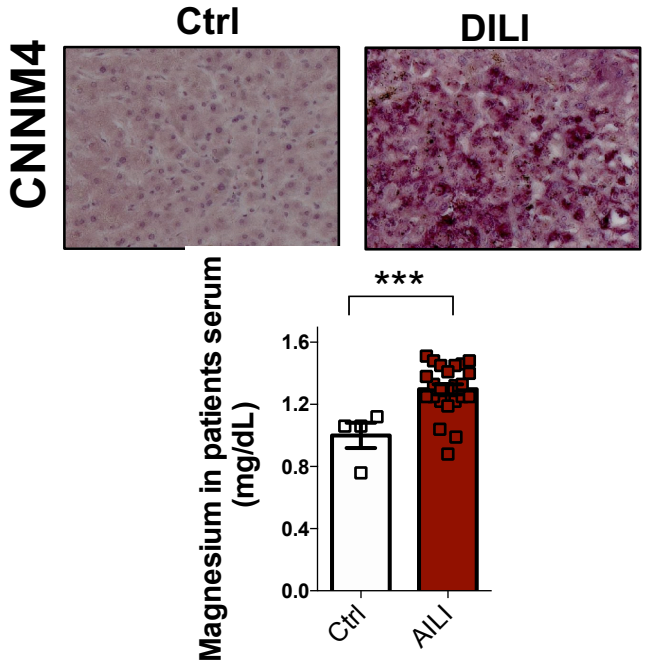
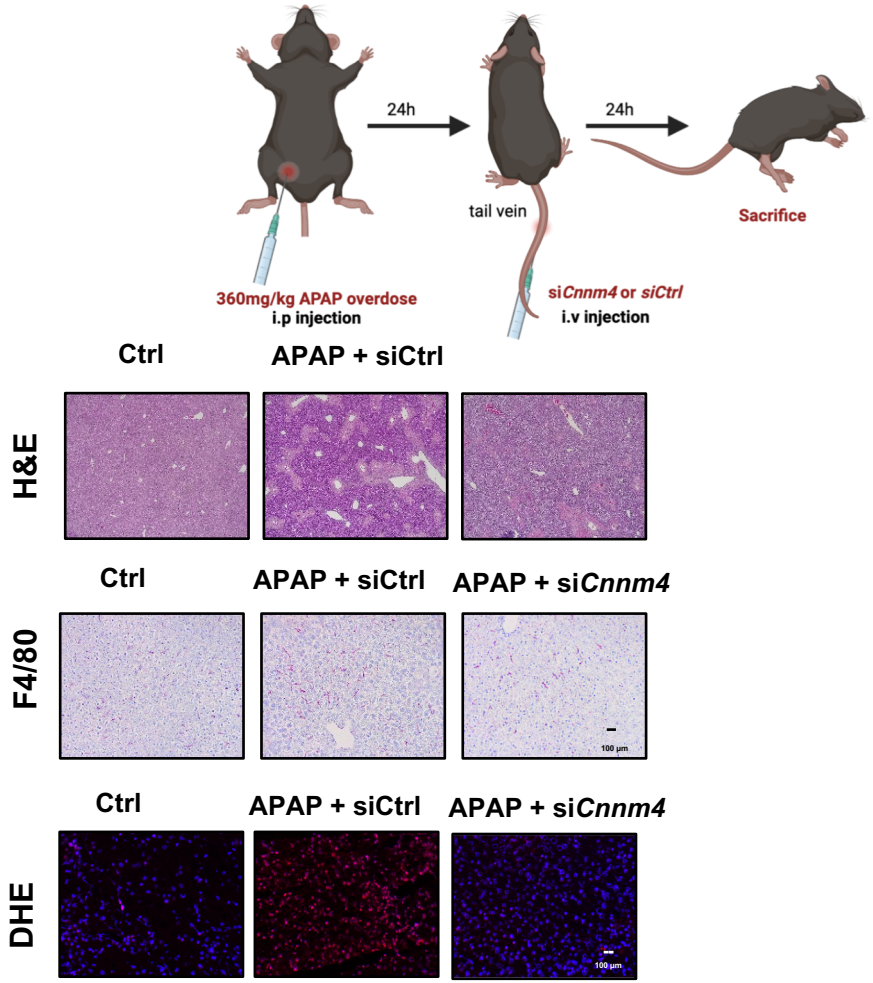
Hepatic CNNM4 expression is increased in AILI which is also correlated with Mg^{2+} levels in the serum in preclinical animal models



Hepatic CNNM4 expression is increased in AILI in patients which is also correlated with Mg^{2+} levels in the serum



González-Recio et al. Nature Communications inpress



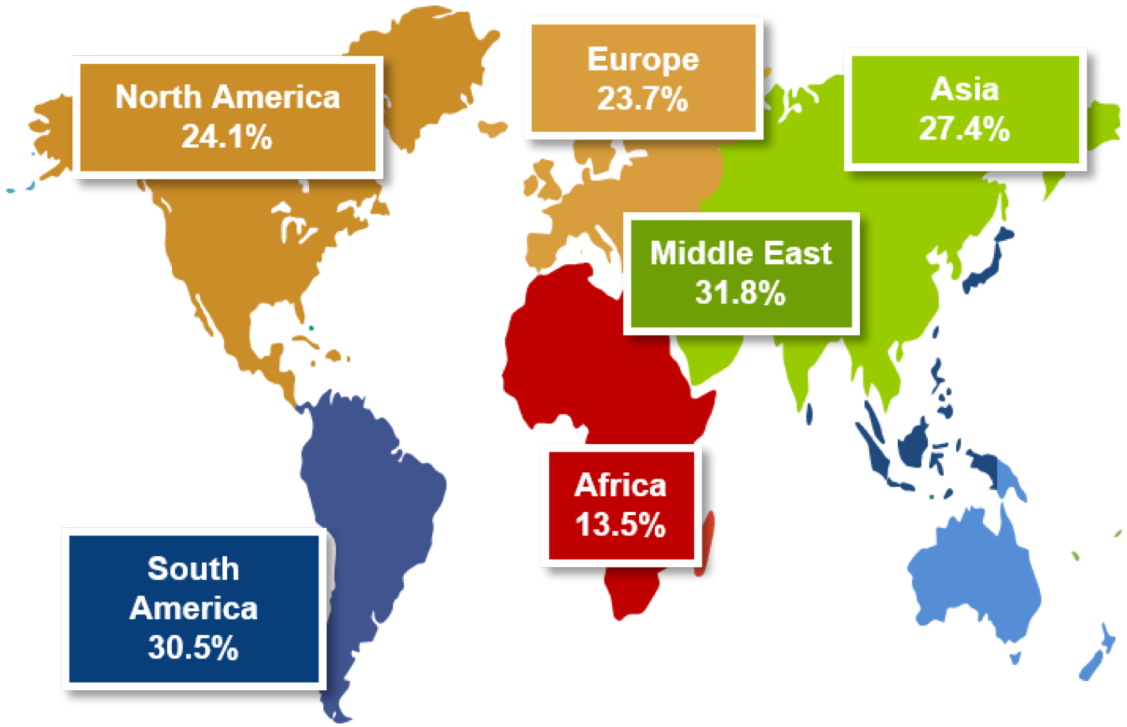
Silencing *Cnnm4* reduced necrotic areas, number of macrophages and reactive oxygen species

0.2 The Product: Current Status of the Development – NASH/NAFLD

World population: **7.5 billion**

People with NAFLD: **1.8 billion**

Prevalence rate: **~25%**



THE LANCET
Gastroenterology & Hepatology

ARTICLES | VOLUME 7, ISSUE 9, P851-861, SEPTEMBER 01, 2022

The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis

Kiarash Riazi, MD • Hassan Azhari, MD • Jacob H Charette, MD • Fox E Underwood, MSc • James A King, MSc • Elnaz Ehteshami Afshar, MD • et al. Show all authors

Published: July 04, 2022 • DOI: https://doi.org/10.1016/S2468-1253(22)00165-0 • Check for updates

Estimated NAFLD prevalence 2036 -> 37.8%

Younossi, Z, et al. Hepatology 2018; 69:2672-2682.

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J Hepatol. 2021 Jul;75(1):34-45. doi: 10.1016/j.jhep.2021.01.043. Epub 2021 Feb 9.

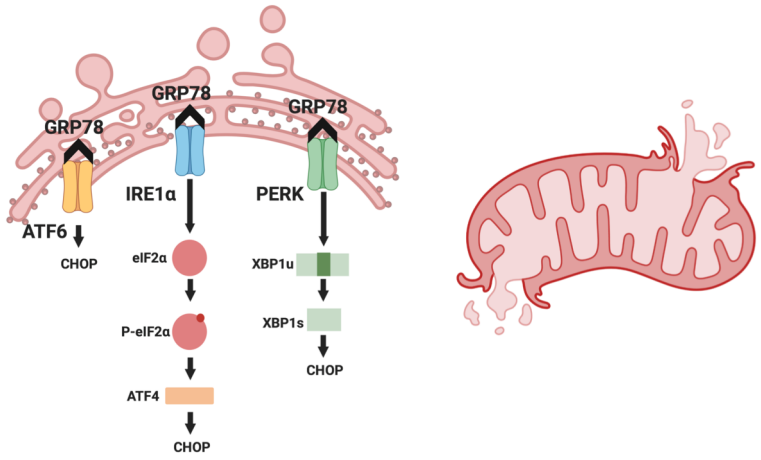
Magnesium accumulation upon cyclin M4 silencing activates microsomal triglyceride transfer protein improving NASH

Jorge Simón [1], Naroa Goikoetxea-Usandizaga [2], Marina Serrano-Maciá [2], David Fernández-Ramos [3], Diego Sáenz de Urturi [4], Jessica J Gruskos [5], Pablo Fernández-Tussy [2], Sofía Lachiondo-Ortega [2], Irene González-Recio [2], Rubén Rodríguez-Agudo [2], Virginia Gutiérrez-de-Juan [2], Begoña Rodríguez-Iruretagoyena [2], Marta Varela-Rey [1], Paula Gimenez-Mascarell [2], María Mercado-Gomez [2], Beatriz Gómez-Santos [4], Carmen Fernandez-Rodriguez [2], Fernando Lopitz-Otsoa [6], Maider Bizkarguenaga [6], Sibylle Dames [7], Ute Schaeper [7], Franz Martin [8], Guadalupe Sabio [9], Paula Iruzubieta [10], Javier Crespo [10], Patricia Aspichueta [11], Kevan H-Y Chu [5], Daniela Buccella [5], César Martín [12], Teresa Cardoso Delgado [2], Luis Alfonso Martínez-Cruz [2], María Luz Martínez-Chantar [13]

Affiliations + expand

PMID: 33571553 PMCID: PMC8217299 DOI: 10.1016/j.jhep.2021.01.043

Free PMC article



Perturbations on Mg^{2+} homeostasis

↓

Altered mitochondria-ER crosstalk

↓

Common feature and driver for the progression of chronic and acute **liver diseases**

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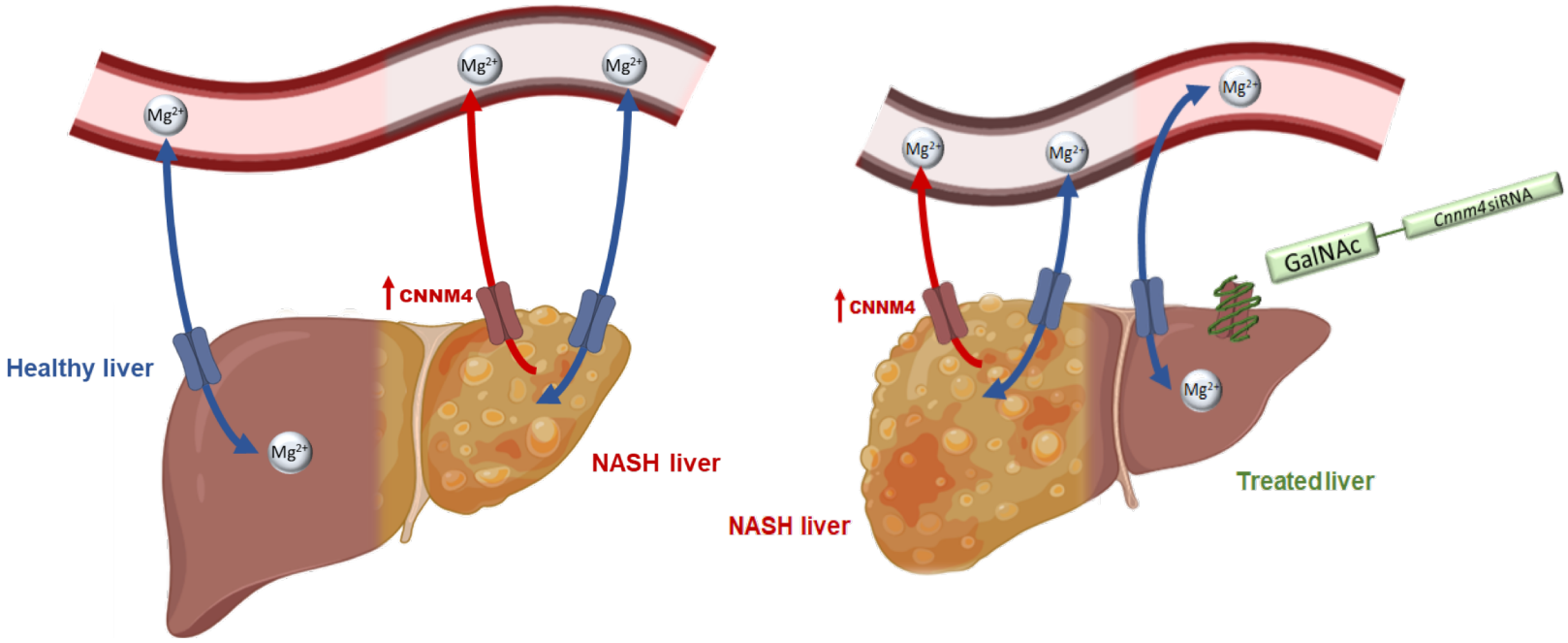

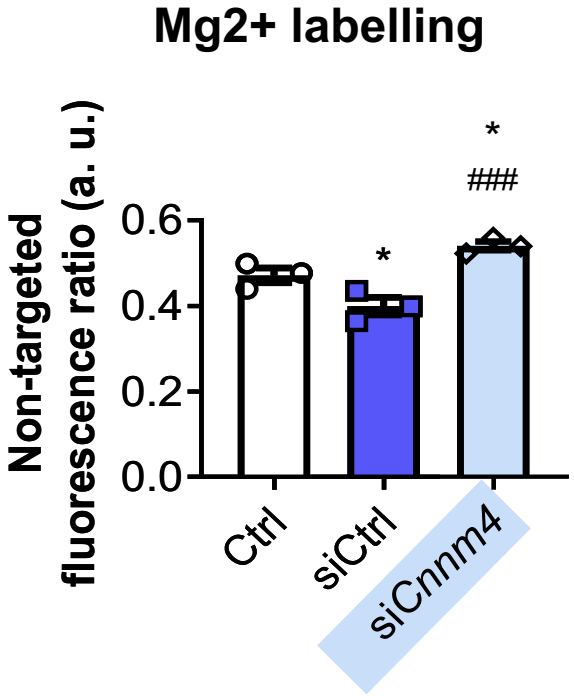

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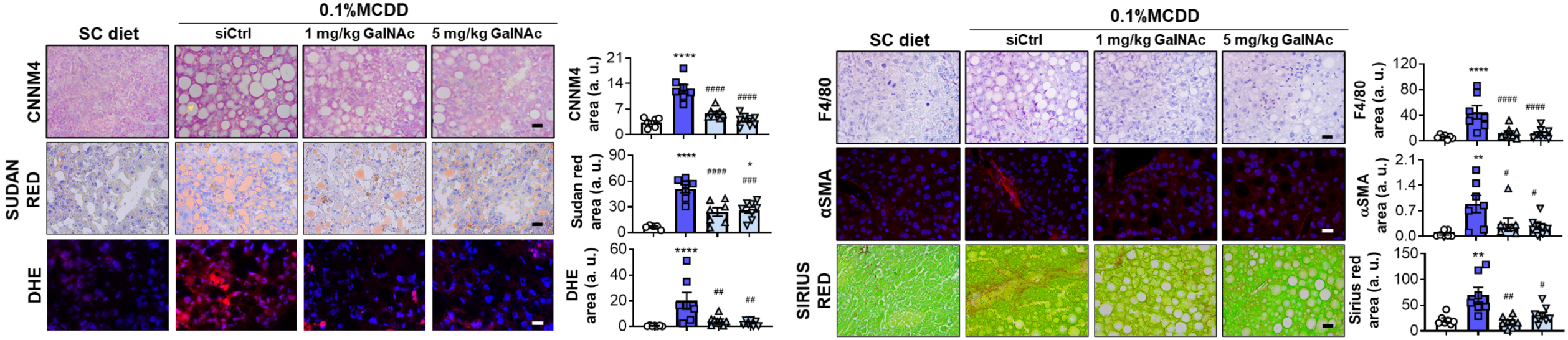
Liver

The conjugation of a siRNA against *Cnnm4* with a N-acetylgalactosamine (**GalNAc-siRNA**) allows a stable delivery to the liver with a **s.c.** administration.



The treatment of mice with **GalNAc-siRNA** leads to a CNNM4 downregulation and the subsequent reduction of NASH hallmarks: lipid accumulation (sudan red), inflammation (DHE & F4/80) and fibrosis development (αSMA & sirius red)

The technology is being protected by **two patent filings (1/2)**

The technology is protected by PCT Patent filing through the European Patent Office with a priority date of 26 of November 2018 and a publication date of 4 of June 2020.

Publication Number	WO/2020/109316
Priority Data	EP18382853.2 – 26 November 2019
Applicants	ASOCIACIÓN CENTRO DE INVESTIGACIÓN COOPERATIVA EN BIOCIENCIAS (CIC-BIOGUNE)
Inventors	SIMÓN, Jorge MARTÍNEZ CHANTAR, María Luz MARTÍNEZ DE LA CRUZ, Alfonso
Title	METHODS FOR DIAGNOSING AND/OR TREATING ACUTE OR CHRONIC LIVER, KIDNEY OR LUNG DISEASE
Current status	National phases: Canada, Singapore, Japan, Israel, Mexico, United States of America, Brazil, Republic of Korea, Australia, Europe, Russia and China.

Europäisches Patentamt
European Patent Office
Office européen des brevets

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The technology is being protected by **two patent filings (2/2)**

The technology is protected by PCT Patent filing through the European Patent Office with a priority date of 27 of May 2020 and a publication date of 2 of December 2020.

Publication Number	WO/2021/239825
Priority Data	EP20382449.5 – 27 May 2020
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Title	NUCLEIC ACIDS FOR INHIBITING EXPRESSION OF CNNM4 IN A CELL
Current status	The patent is currently in PCT period (Patent Cooperation Treaty)

Europäisches Patentamt
European Patent Office
Office européen des brevets

CIC bioGUNE
CENTER FOR COOPERATIVE RESEARCH IN BIOSCIENCES

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LIVER DISEASE LAB

0.2 The Product: Pitfalls

There are several high risks to take into consideration for the development of the GalNac-siRNA targeting CNNM4:

Risk	Probability	Type	Mitigation Plan
No added value recognition	**Low**	**Clinical**	The group's expertise in the area of Liver diseases as well as the Proof of Concept results already obtained show the potential of the GalNaC-siRNA-CNNM4 drug.
Not reach the expected clinical performance	**Medium**	**Technical**	Clinical trials are needed to prove the Safety and Efficacy of the drug. Although clinical performance cannot be evaluated until the realization of such clinical trials. Based on Bibliography and Proof of Concept results, clinical trials will be directed into a coherent indication. Moreover, Clinical trials will be correctly designed.
No Freedom to Operate	**Low**	**Business**	The project already counts with two patents that protect the asset: WO/2020/109316 & WO/2021/239825. GalNAC IP negotiated. FtO analysis by Q42022
Not being able to attract enough funding to advance with the drug development	**Medium**	**Business**	The project will have to raise money to complete all the steps to bring the drug into the market. The group is committed into creating a Spin-Off, fact that would broaden the funding opportunities according to the nature of a private company.
No compliance with the regulatory requirements needed for a GalNac-siRNA	**Low**	**Regulatory**	All regulatory requirements will be studied in collaboration with a Regulatory consultants with expertise in the area. Contacts with AEMPS & EMA will be executed to ensure the compliance with the needed regulatory requirements.

Strategic Regulatory Roadmap Support

As it is known, the development of therapies usually entails many challenges which include a **robust regulatory framework which development must comply with.**

In order to facilitate the process and its adequacy to regulatory standards that eventually would foster approval in a timely and cost-effective manner, **a regulatory roadmap is going to be performed in the pre-clinical stages at the end of 2022-beginning of 2023.**

The purpose of this roadmap is to **establish the regulatory milestones to be achieved by CiC bioGUNE in the development of the siRNA molecule, within the indication of ALD and fibrosis** in order to comply with regulatory demands and facilitate future commercialization and access to patients.

Initial Regulatory Assistance for the Development of the siRNA for CIC bioGUNE

Definition of **Regulatory Pathway applicable, documentation, and key milestones** to proceed

Identification **of key processes with authorities** to validate results and facilitate development

General recommendations on **CMC and Manufacturing**

Strategic preclinical development: pre-clinical studies to be performed in pharmacology, pharmacodynamics and toxicity to advance to FiH trial

Regulatory Milestones During Development

Key Regulatory Milestones

The following information shows the key milestones to be obtained for regulatory development within the European Union up to a First in Human Trial.

- **Regulatory Roadmap** **Execution start in Q12023**
- Adequate **Pre-Clinical** **Studies defined by Q12023**
- Product **manufacturing under GMPs** conducted in parallel to pre-clinical testing and should be ready to initiate FiH trial. **Provider idenntified**
- **IMPD** and **IB** Writing 6 months prior to initiation of Clinical Trial Application
- **Clinical Trial Application**

A strategic regulatory roadmap will be performed to define the regulatory milestones and pre-clinical strategy to successfully achieve a Phase I study with the molecule.

Preclinical validation of own MCH003 (patenting Pending) toward another undisclosed target for:

1. Hepatocellular Carcinoma
2. Glucose Metabolism

Scouting and in-licensing of new hit compounds for new potential targets

New Partnership and developments for GalNAC like vehicle

In house new target and vehicle validation in animal models

Thank you for your time

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